[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, INSTITUTE OF TECHNOLOGY, UNIVERSITY OF MINNESOTA]

Quenching of the Fluorescence of Chlorophyll a Solutions[1]

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There is no direct, general way of determining the nature of the primary act[3] of a photochemical reaction in which the light is absorbed by a complex molecule. One indirect method, of obtaining evidence related to the primary act, is to study the fluorescent yield of the complex molecule under a variety of conditions. As part of an attempt to investigate the primary act in the process of photosynthesis, we have studied the quenching of the fluorescence of chlorophyll a solutions by a variety of added substances.

While it has been known for some years that the fluorescence of chlorophyll solutions is quenched by oxygen,[4,5,6] by quinone,[6] by nitrobenzene[7] and possibly by other substances,[4b,6] the measurements upon which this information was based are largely semi-quantitative in nature and were made with either crude plant extracts[4b] or with mixtures of chlorophyll a and b.[6] In the present experiments, pure chlorophyll a and carefully purified solvents and reagents were used.

Experimental Methods and Materials

The Apparatus.—The fluorimeter and fluorescence cells used in these experiments have been described recently.[8] The exciting light was either of λ 4358 Å. or a narrow band having its maximum at 6450 Å. The former was isolated from the radiation of a 100-watt G. E. AH-4 mercury arc by means of Corning filters no. 5850 and 3389 and 3 cm. of 5% copper sulfate solution. The band at λ 6450 Å. was isolated from the radiation of a 1000-watt incandescent[9] projection lamp by 3 cm. of 5% copper sulfate solution and a Farrand interference filter no. 1039. The photocell was covered by a Wratten filter no. 88, which absorbs all radiation of wave lengths less than 6800 Å.

Routine Procedure.—A known volume of a stock solution of chlorophyll a in ether was pipetted into the fluorescence cell, an excess of the solvent was added and the ether and excess solvent were pumped off until the solution had reached a standard volume, corresponding to a chlorophyll concentration of $1.2 \times 10^{-5}m$. The cell was then placed in the clamp of the fluorimeter and allowed to remain (in the dark) until it had returned to room temperature. The fluorescence intensity, I_0, of the unquenched solution was then measured. Following this, the cell was opened and a known amount of quencher and an excess of solvent were added. The excess solvent was then pumped off to restore the solution to its standard volume, and the fluorescence intensity determined as before. When gaseous quenchers were used, the purified gas at a known total pressure was admitted to the chlorophyll solution, contained in the evacuated cell. The cell was shaken and allowed to stand in contact with the gas (at a known pressure) to ensure the attainment of equilibrium at room temperature.

Purification of Materials.—Chlorophyll a was isolated from fresh spinach by a modification[10] of the method of Zscheile and Comar.[11] It was dissolved in freshly purified ether, and its stock solutions were stored in the dark at approximately 0°. The purity and concentration of these solutions were determined spectrophotometrically with a Beckman spectrophotometer.[12]

The purification of the methanol has been described elsewhere.[13] Ether was treated with several portions of concentrated C. P. sulfuric acid, then allowed to stand over sodium until the reaction was apparently complete, and finally distilled in an all-glass still. Acetone was allowed to stand over anhydrous potassium carbonate for several weeks, and was then distilled through an efficient, packed column. Butanol and octanol were dried with anhydrous calcium sulfate and then distilled. Reagent grade benzyl alcohol was used without further purification.

The reagents which were tested for quenching action were either known to be of high purity or were carefully purified. Methyl red, β-nitroso-α-naphthol, *p*-quinone, 2,6-diaminopyridine, trinitrotoluene and *m*-dinitrobenzene were recrystallized from the appropriate solvents and dried in vacuum desiccators. Nitropropane, phenylhydrazine and benzaldehyde were dried and then vacuum distilled. Aniline was recrystallized as the hydrochloride, treated with potassium hydroxide, dried with solid potassium hydroxide, and carefully fractionated in vacuum. *p*-Aminophenol was purified by treating an aqueous solution of the hydrochloride with saturated sodium thiosulfate, filtering under an inert atmosphere, then recrystallizing from 50% alcohol twice and from absolute alcohol once. Nitrobenzene was recrystallized from absolute alcohol (by refrigeration) three times, dried with calcium chloride, and twice vacuum fractionated. Since it was suspected that dimethylaniline might contain traces of oxidation products it was purified by three separate methods. One sample was prepared by fractionally crystallizing from the liquid; the second, by fractional crystallization from 80% alcohol and then from absolute alcohol, followed by vacuum distillation; and the third, by crystallizing as the oxalate, liberating the base with alkali, drying with solid potassium hydroxide, and vacuum distilling. The three samples had similar properties. Nitrous oxide and oxygen were taken from commercial cylinders and used without further purification. Nitric oxide was taken from a commercial cylinder, washed with sulfuric acid and water, and then dried. Carbon monoxide was prepared from

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(3) W. A. Noyes and P. A. Leighton, "The Photochemistry of Gases," Reinhold Publishing Corp., New York, N. Y., 1941, p. 88.

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(6) V. Evstigneev and A. Krasnovskii, *Doklady Akad. Nauk S. S. S. R.*, **60**, 623 (1948).

(7) O. Biermacher, Thesis, Univ. of Fribourg, 1936.

(8) R. Livingston, W. Watson and J. McArdle, THIS JOURNAL, **71**, 1542 (1949).

(9) J. McBrady and R. Livingston, *J. Phys. Coll. Chem.*, **52**, 662 (1948)

(10) R. Livingston, D. Sickle and A. Uchiyama, *ibid.*, **51**, 777 (1947).

(11) F. Zscheile and C. Comar, *Botan. Gaz.*, **102**, 463 (1941).

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(13) R. Livingston and R. Pariser, THIS JOURNAL, **70**, 1510 (1948).

TABLE I

SUMMARY OF QUENCHING DATA

Quencher	Solvent	t, °C.	λ, Å.	n	Limits of [Q]	$[Q]_{1/2}$	k_1	k_2	k	$k^2/8$
Chloranil	Me_2CO	25	6450	9	0.0015–0.010	0.0050	175	4700	180	4050
Quinone	Me_2CO	25	6450	3	.011 – .032	.0081	143	260	111	1540
Quinone	MeOH	24	6450	11	.0050– .052	.0096	120	300	93	1090
Methyl red	MeOH	24	6450	3	.0029– .0076	(.0088)	101	1360	102	1300
Trinitrotoluene	MeOH	24	6450	16	.0009– .045	.0100	90	1020	90	1010
Trinitrotoluene	MeOH	27	4358	11	.0009– .027	.0098	113	100	92	1050
m-Dinitrobenzene	MeOH	24	6450	8	.0012– .061	.0122	68	820	74	680
m-Dinitrobenzene	MeOH	29	4358	10	.0012– .022	.0110	77	1140	82	840
Duroquinone	MeOH	22	6450	6	.0094– .037	.0116	81	580	77	750
β-Nitroso-α-naphthol	MeOH	24	6450	7	.007 – .044	.0140	62	580	64	520
β-Nitrostyrene	MeOH	27	4358	6	.0034– .034	.0170	59	150	34	370
Nitric oxide	EtOH	23	4358	4	.0036– .0094	(.0178)	43	1440	51	320
β-Nitro-β-methylstyrene	MeOH	29	4358	4	.0038– .023	.022	41	250	42	215
Oxygen	EtOH	27	4358	5	.0014– .0082	(.023)	35	880	39	195
Nitrobenzene	MeOH	22	4358	12	.0045– .16	.034	36	3	26	87
β-Nitro-β,γ-hexene	MeOH	27	4358	7	.019 – .19	.064	12	42	14.0	25
p-Aminophenol	MeOH	28	4358	3	.074 – .16	.138	7.2	1.1	6.5	5.3
Phenylhydrazine	Et_2O	22	4358	8	.13 – .47	.151	8.4	−3	6.0	4.5
Phenylhydrazine	MeOH	22	4358	9	.13 – .78	.31	3.7	−0.4	3.0	1.1
Dimethylaniline	MeOH	23	4358	8	.018 – .40	(.42)	2.4	−0.5	2.1	0.55
2-Phenyl-3-nitrobicyclo-[1,2,2]heptene-5	MeOH	27	4358	7	.055 – .22	(.61)	1.6	−0.3	1.5	0.27
2,6-Diaminopyridine	MeOH	23	4358	9	.067 – .58	(.62)	1.4	0.3	1.45	0.26

oxalic acid and sulfuric acid and was washed with potassium hydroxide solution, and then dried. Carbon dioxide was prepared by evaporating Dry Ice. Samples of carefully purified duroquinone and of chloranil were kindly furnished by Dr. Lee I. Smith. Samples of pure β-nitrostyrene, β-nitro-β-methylstyrene and β-nitro-β,γ-hexene were prepared and furnished by Mr. J. Bleasdale. A sample of 2-phenyl-3-nitrobicyclo[1,2,2]heptene-5 (b. p. 136–138° at 1–2 mm.) was prepared and supplied by Mr. W. H. Hunter. All other compounds tested were of reagent grade and were used without further purification.

Experimental Results

The compounds examined for their efficiency have been grouped, for sake of convenience, into "quenchers" and "non-quenchers." The results obtained with the quenchers are summarized in Table I and in Figs. 1, 2 and 3. The first four columns of Table I list, respectively, the quencher, the solvent, the temperature at which the experiments were run, and the wave length of the exciting light. Corresponding to each series, the number of measurements are given in column five and the highest and lowest concentration of the quencher in column six. The values of the quantity $[Q]_{1/2}$, listed in column seven, are the molarities of the quencher necessary to reduce the intensity of fluorescence to half of its unquenched value. The numbers in this column which are in parentheses were obtained by extrapolation. The absorption spectra of each of the quenchers was measured over the range λ 3200 to 6900 Å. with the Beckman spectrophotometer. Where necessary, the observed values of I have been corrected for the internal filter action of the quencher. Such corrections were usually small, seldom exceeding 10%. The values of I_0/I, and of $(I_0/I - 1)/[Q]$, plotted in Figs. 1, 2 and 3 and summarized in Table I were so corrected.

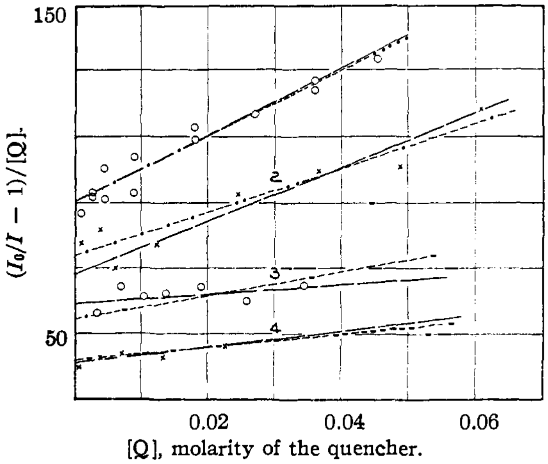

Fig. 1.—Typical quenching results: 1, trinitrobenzene; 2, *m*-dinitrobenzene; 3, β-nitrostyrene; 4, β-nitro-β-methylstyrene.

In general the absorption spectra of the quenched chlorophyll was not determined. However, in two cases (nitrobenzene and phenylhydrazine) where the absorption spectrum of chlorophyll a in the presence of approximately 0.3 *m* quencher was measured carefully by W. Watson and J. McArdle,[14] it was not detectably altered.[15]

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[14] Unpublished measurements.

[15] This is in marked contrast to the effect of phenylhydrazine upon the absorption spectrum of chlorophyll b, which is distinctly changed.[10]

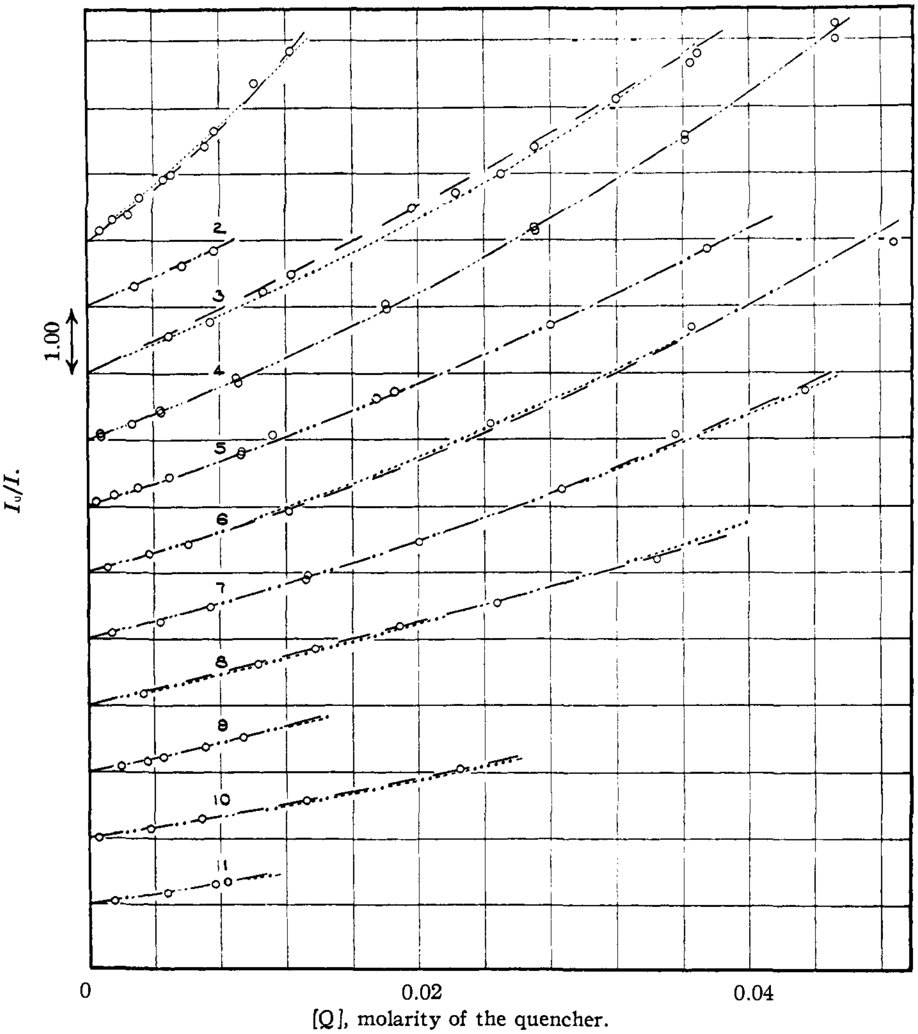

Fig. 2.—Summary of quenching data: 1, chloranil; 2, methyl red; 3, quinone; 4, trinitrotoluene; 5, duroquinone; 6, *m*-dinitrobenzene; 7, β-nitroso-α-naphthol; 8, β-nitrostyrene; 9, nitric oxide; 10, β-nitro-β-methylstyrene; 11, oxygen.

The reversibility of the quenching process has been proven definitely only for the two gaseous quenchers. Removal of dissolved oxygen or nitric oxide from a partially quenched chlorophyll solution restores its fluorescence to its original value. In a few preliminary experiments (chiefly with phenylhydrazine), the effect of diluting a partially quenched solution with solvent was studied. When proper allowance is made for the dilution of the chlorophyll, the observed change in the fluorescence intensity corresponds to completely reversible quenching.

The present results are in general agreement with those reported by previous workers. The per cent. quenching produced by saturating an ethanolic solution with oxygen is reported as 35, 20, and 30%, respectively, by Franck and Levi,[4b] Evstigneev and Krasnovskii[6] and the present authors. However, the half-quenching concentration $[Q]_{1/2}$, listed in Table I, for methanolic solutions of quinone, is about threefold smaller than that reported by Evstigneev and Krasnovskii[6] for solutions in ethanol. The quenching reported by them for hydroquinone is so weak as to be consistent with the present classification of it as a "non-quencher." We have also confirmed their finding that sodium iodide and ascorbic acid are non-quenchers.

The variation of the intensity, *I*, of the fluorescence as a function of the concentration [Q] of the quencher corresponds approximately (particularly for low values of the concentration) to the Stern–Volmer equation.

$$I_0/I = 1 + k_1[Q] \qquad (1)$$

where I_0 is the intensity of the unquenched fluorescence under the conditions of the experiment. At high or moderate concentrations the data show a definite departure from this relation, but can be represented within the limits of

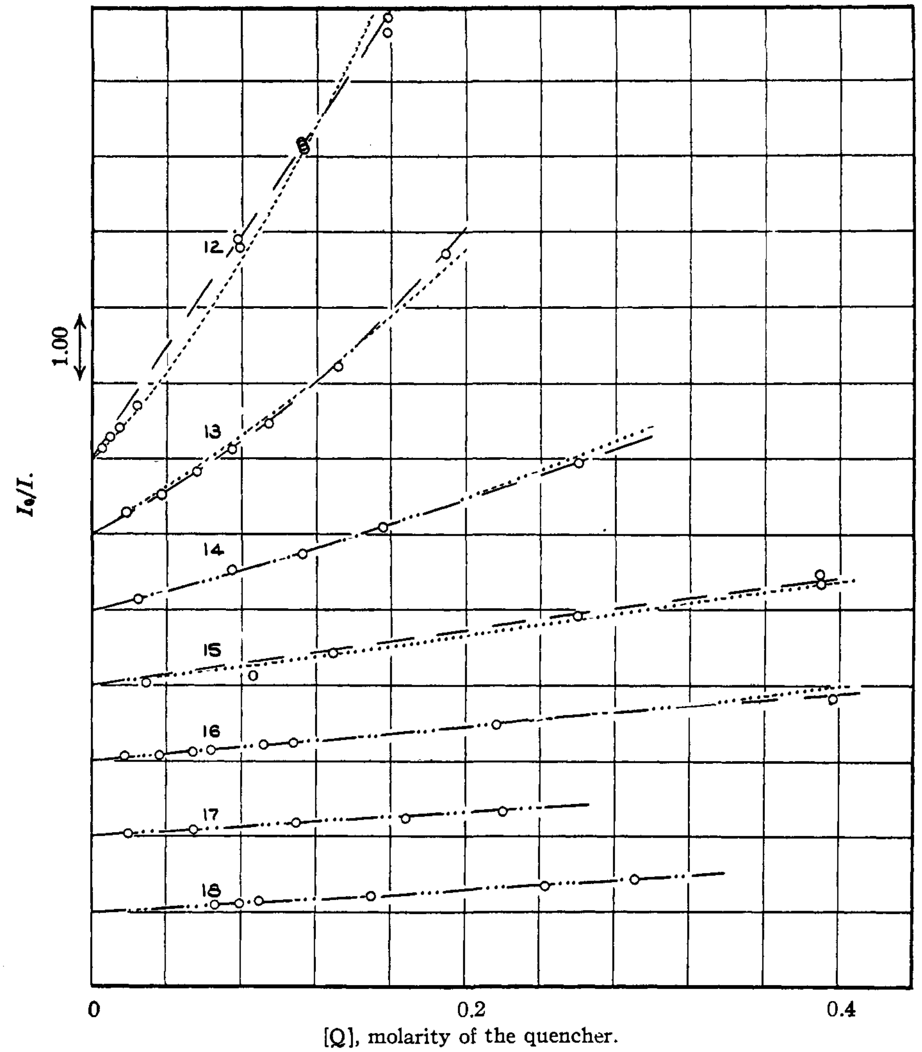

Fig. 3.—Summary of quenching data: 12, nitrobenzene; 13, β-nitro-β,γ-hexene; 14, p-aminophenol; 15, phenylhydrazine; 16, dimethylaniline; 17, 2-phenyl-3-nitrobicyclo[1,2,2]heptene-5; 18, 2,6-diaminopyridine.

experimental error by the modified relation which was recently discussed by Rollefson and Boas.[16]

$$I_0/I = 1 + k_1[Q] + k_2[Q]^2 \qquad (2)$$

Values of k_1 and k_2, which were obtained by an unweighted least squares calculation, are listed in columns 8 and 9 of Table I. Examination of these data indicates that k_1 and k_2 are symbatic. This fact suggested that possibly an equation similar to equation 2, but containing only one adjustable constant, might fit the present experimental data within its limits of accuracy. The following expression (equation 3) represents the empirical results within a reasonable estimate of their precision[17]

$$I_0/I = 1 + k[Q] + k^2/8[Q]^2 \qquad (3)$$

The values of k and $k^2/8$ listed in columns 10 and 11 of Table I were obtained by fitting the empirical data to equation 3 using the "method of averages."

Figure 1 illustrates the marked departure from the Stern–Volmer law (equation 1). Values of $(I_0/I - 1)/[Q]$ are plotted against [Q] for four nitro compounds which are strong quenchers. The intercepts of the straight lines on the ordinate axis correspond to the values of k_1, and the slopes of the lines are equal to k_2. The dash lines are plots of equation 2, and the dot lines, equation 3.

All of the data[18] upon which Table I is based are plotted in Figs. 2 and 3. To avoid exaggeration of the errors in the low-quenching range, values of I_0/I (rather than $(I_0/I - 1)/[Q]$) have been plotted against [Q]. The dash-line curves are plots

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[16] G. Rollefson and H. Boas, *J. Phys. Coll. Chem.*, **52**, 518 (1948).

[17] A slightly better fit can be obtained by using the relation

$$I_0/I = 1 + k[Q] + k^2/8[Q]^2 - k^3/70[Q]^3$$

However, since the improvement in fit is slight and may not be significant, it appeared advantageous to retain the simpler expression (equation 3) in the present discussion.

[18] Where more than one solvent or wave length were used for a compound, only the data corresponding to methanol and to 6450 have been plotted.

of equation 2 and the dotted curves of equation 3. In these figures, k_1 is a measure of the slope and k_2 of the curvature. To avoid the overlapping of lines and points, a new origin has been taken for each case.

The determination that certain classes of compounds are non-quenchers is of comparable interest to the identification of efficient quenchers. Table II lists those substances whose quenching efficiency was studied and proved to be small or negligible. Of these, only phenylhydroxylamine has an appreciable quenching action. Even in this case, the slight effect which was observed may have been due to traces of impurities, since the sample was not especially purified. In a few cases (notably benzaldehyde, benzoic acid and sodium methoxide), the quencher and chlorophyll solutions were simultaneously evacuated in separate compartments of the same vessel, and the fluorescence was measured immediately after mixing the solutions. This technique was necessary since chlorophyll undergoes fairly rapid, irreversible changes in the presence of these reagents. In addition to the substances listed in Table II, the following compounds have been shown by earlier workers to be non-quenchers: sodium iodide,[6] potassium iodide,[6] ascorbic acid[6] and allylthiourea.[19]

TABLE II

EFFECT OF NON-QUENCHERS

Reagent	Solvent	[Q]molar	I_0/I
Nitropropane	MeOH	0.09	1.00
Nitropropane	MeOH	.29	1.02
Butyl nitrate	MeOH	.38	1.02
Butyl nitrite	Me_2CO	.11	1.01
Phenylhydroxylamine	MeOH	.02	1.02
Phenylhydroxylamine	MeOH	.07	1.05
Aniline	Et_2OH	.16	1.02
Hydrazine	Me_2CO	1.05	1.0
Urethan	Me_2CO	0.07	1.0
Thiourea	Me_2CO	.02	1.0
2-Aminopyridine	MeOH	.08	1.00
Phenylurea	MeOH	.05	1.01
Urea	MeOH	.19	1.00
Guanidine carbonate	MeOH	(Saturated)	1.0
Phenol	MeOH	0.09	1.0
Hydroquinone[a]	Me_2CO	.03	1.0
Phenolphthalein	MeOH	.04	1.0
Dimethylglyoxime	MeOH	.07	1.0
t-Hexyl mercaptan	MeOH	.11	1.00
Benzaldehyde	MeOH	.38	1.0
Benzoic acid	Me_2CO	.08	1.0
Camphor	MeOH	.15	1.0
Boric acid	MeOH	(Saturated)	1.00
Sodium methoxide	MeOH	0.05	1.0
Sodium cyanide	MeOH	.15	1.01
Sodium azide	MeOH	.11	1.0
Nitrous oxide	EtOH	(605 mm.)	1.0
Carbon dioxide	EtOH	(576 mm.)	1.0
Carbon monoxide	MeOH	(640 mm.)	1.00
Potassium thiocyanate	MeOH	0.05	1.02
Potassium thiocyanate	Me_2CO	.004	0.92

[a] The measurements of Evstigneev and Krasnovskii[6] indicate that hydroquinone at much higher concentrations has some quenching action, but is less efficient than 2-diaminopyridine, the weakest quencher in Table I.

The increase in fluorescence intensity produced by the addition of sodium thiocyanate to solutions of chlorophyll in acetone, is probably related to the activation[8] of the fluorescence of chlorophyll dissolved in hydrocarbons. The fluorescence of chlorophyll dissolved in acetone is distinctly less (by a factor of about 0.7) than the fluorescence of methanolic solutions of chlorophyll.

Discussion

As is illustrated by Figs. 2 and 3, the experimental data are consistent with either equation 2 or 3. Since equation 2 contains an additional adjustable constant it can be fitted to a given set of data more exactly; however, this improvement does not appear to be significant for the present data. Both equations are empirical and their constants have been adjusted to correspond to the available data. Furthermore, equation 3 has been only shown to hold at constant temperature and viscosity for the quenching of chlorophyll a by a limited group of quenchers.

Assuming that the observed quenching is the result of diffusional and static processes, Frank and Wawilow[20] derived an equation of the form

$$\frac{I_0}{I} = e^{\omega[Q]}\left(1 + \frac{AT}{\eta}[Q]\right)$$

where A and ω are constants whose values depend chiefly upon the properties of the quencher and the fluorescent molecule, and where T and η are the temperature and the coefficient of viscosity of the solvent, respectively. When the quantity $\omega[Q]$ is less than unity, the exponential may be represented as a series, and to a good approximation

$$\frac{I_0}{I} \simeq 1 + \left(\frac{AT}{\eta} + \omega\right)[Q] + \left(\frac{AT}{\eta} + \frac{\omega}{2}\right)\omega[Q]^2 \quad (4)$$

Comparing equations 2 and 4 and making the simplifying assumption that $AT/\eta + \omega \simeq AT/\eta + \omega/2$, we obtain $k_2/k_1 \simeq \omega$. For the strong and moderate quenchers ($(k_1) \geqslant 10$ l./mole) the empirical values of k_2/k_1 lie between 30 and 0.8 l./mole or between 5×10^{-20} and 1.3×10^{-21} cc./molecule. The corresponding radii are 23 and 7 Å. Since ω is defined as the volume within which static quenching will occur, those numerical values are within the expected range[21] and accordingly support the (limited) validity of equation 4.

If it be assumed that A and ω are functions of the quencher and fluorescent substance only, an independent estimate of the value of ω can be obtained from a series of measurements using the same quencher and fluorescent substance but different solvents. Preliminary measurements with nitrobenzene and chlorophyll a yielded the

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(19) Unpublished experiments of Dr. W. Watson.

(20) J. Frank and J. Wawilow, *Z. Physik*, **69**, 100 (1931).

(21) Compare Förster, *Ann. Physik*, **2**, 55 (1948).

following values of k_1 in the solvents indicated: 36 in methanol, 25 in ethanol, 17 in butanol, 10.5 in octanol, 5.1 in benzyl alcohol. If these values and the corresponding values of η for the solvents are substituted in the relation

$$k_1 = AT/\eta_1 + \omega \qquad (5)$$

a (rather uncertain) value of ω about tenfold greater than that found by the preceding method is obtained. Furthermore, the data do not appear to conform to equation 5; the value of k_1 probably being dependent upon other properties of the solvent in addition to its viscosity.[22] Additional and more precise data will be required to decide whether equation 5 can be applied to this system.

It was predicted[5] by Professor James Franck that complex molecules should show an "unquenchable" fluorescence, persisting at high concentrations of the quencher or even where the quencher and fluorescent molecule form a complex. To test this prediction, measurements of I/I_0 were made in methanolic chlorophyll solutions, containing nitrobenzene at concentrations as great as 4.4 *m*. Within the limits of precision of the measurements, the data can be fitted by the following equation where δ = 0.003.

$$\frac{I}{I_0} = \frac{1 - \delta}{k_1[\mathrm{Q}] + k_2[\mathrm{Q}]2} + \delta$$

Although special precautions were taken to minimize persistent errors, the value of δ is little larger than the inherent uncertainty of the measurements. We must conclude, therefore, that while these experiments appear to indicate the existence of weak (between 0.4 and 0.2%) unquenchable fluorescence, this existence has not been proven.

The strong and moderate quenchers constitute an interesting series of compounds. Most measurements on fluorescence quenching in solution have been made with uranyl ion, and with fluorescein and rhodamine dyes[16,22] which are quenched by such reducing agents as aniline and iodide ion. In contrast to this all of the compounds which have been demonstrated to quench efficiently the fluorescence of chlorophyll are oxidizing agents. In addition to quinones, aryl nitro compounds and olefinic nitro compounds, oxygen, nitric oxide, β-nitroso-α-naphthol and the azo dye, methyl red, are strong or moderate quenchers.

This list of quenchers is strikingly similar to the list of compounds which are known to be effective inhibitors or retarders of polymerization. Quinones, arylnitro compounds,[23,24] β-nitrostyrene,[25] oxygen,[23] nitric oxide,[26] nitroso-β-naphthol[23,24] and diaminoazomethane[23,24] have been shown effectively to inhibit or retard polymerization reactions. In general there appears to be a qualitative parallelism between the relative effectiveness of these compounds as polymerization inhibitors (or retarders) and as fluorescence quenchers. For example, the effectiveness both as quenchers and as retarders of the nitroaryl compounds increases in the order mononitro, dinitro and trinitro compounds. Nitropropane is not a quencher, and nitromethane is not an inhibitor or retarder. Benzidine has been reported to act both as a quencher[3] and as an inhibitor.[24] Most reducing agents are ineffective for both purposes. However, some aminoaryl compounds have been shown (Table I) to be weak quenchers, while others have been reported[24] to be moderately good inhibitors. Some caution must be used in interpreting the inhibiting action of reducing agents. When polymerization is carried out in the presence of an oxidizing agent, there is always the possibility that the observed inhibition is not due to the reducing agent itself but to some oxidation product of it. Studies of photopolymerization in the absence of oxygen are free from this uncertainty.

The explanation of this marked parallelism between the inhibition of polymerization and the quenching of the fluorescence of chlorophyll is not apparent. The similarities, however, seem much too great to be fortuitous. The rate-determining steps in the quenching of the fluorescence of chlorophyll and in the inhibition of polymerization must either be essentially similar or in some way depend upon the same property of the reagents. In this connection, it is suggestive to consider Rollefson's[21] hypothesis, that the efficiency of a quencher is determined by the similarity of the energy difference between its ground singlet and lowest triplet state to the energy difference between the first excited singlet and lowest triplet state of the fluorescence molecule.

Acknowledgment.—The authors wish to acknowledge the assistance of Miss Thomasine Thomas who prepared the chlorophyll used in these experiments and who made some of the measurements. They are indebted to Dr. B. L. Crawford, Dr. R. Dodson, and Dr. W. Lipscomb for valuable suggestions and criticism and to Dr. R. Parham and Dr. L. I. Smith for making accessible some of the less common organic reagents.

Summary

The fluorescence of chlorophyll a solutions is quenched efficiently by oxidizing agents such as quinones, arylnitro compounds, olefinic nitro compounds, nitrosonaphthol, methyl red, oxygen and nitric oxide. It is also quenched weakly by certain amino-aromatic reducing agents. This list of quenchers and their relative efficiencies corresponds, strikingly, to the series of com-

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[22] Compare K. Hodges and V. LaMer, THIS JOURNAL, **70**, 722 (1948).

[23] F. Bovey and I. Kolthoff, *Chem. Revs.*, **42**, 491 (1948).

[24] S. Foord, *J. Chem. Soc.*, 48 (1940).

[25] G. Schultz and M. Strassberger, *Ber.*, **80**, 232 (1947).

[26] I. Kolthoff, personal communication.

pounds which have been reported[23] as inhibitors and retarders for polymerization reactions.

Quantitatively, the quenching of these solutions corresponds to the modified[21] Stern–Volmer equation $I_0/I = 1 + k_1[Q] + k_2[Q]^2$. Less exactly, but apparently within the limits of experimental error, these data conform to the following empirical, one-constant equation $I_0/I = 1 + k[Q] + 1/8\, k^2[Q]^2$.

MINNEAPOLIS 14, MINNESOTA RECEIVED AUGUST 15, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING OF THE UNIVERSITY OF NEW HAMPSHIRE]

A Polarographic Examination of Diazotized Amines[1]

BY EDWARD R. ATKINSON, HAROLD H. WARREN, PAUL I. ABELL AND RICHARD E. WING

In considering mechanisms by which reducing agents convert diazotized amines to biaryls,[2,3] it was of interest to observe whether there was any difference in polarographic half-wave potentials between those diazo compounds which gave chiefly biaryl and those which gave chiefly azo compounds when treated with cupro-ammonia ion.[2] Polarographic information was also desirable in order that we might resume our study of large-scale electrolytic reduction at a mercury cathode[4] under correct controlled potentials.

We have examined the polarographic behavior of diazo compounds derived from aniline and the three isomeric aminobenzoic acids. No polarographic examination of diazotized amines has been reported previously.[5]

Materials and Procedure

Benzenediazonium chloride was prepared by the method of Pray[6]; the crude salt was washed with anhydrous ether and reprecipitated once again from anhydrous ethyl alcohol by the dropwise addition of ether. After drying the salt was dissolved in ice water to prepare 0.1 *M* stock solutions which were stored at 0° in the dark and used within six hours of preparation. Suitable polarographic curves were obtained from solutions prepared by the direct diazotization of the amine provided that excess nitrous acid was eliminated by addition of urea; such solutions were not used throughout the work because of uncertainties concerning their exact concentration. The solid salt used by us was analyzed for chloride by an adsorption indicator technique[7] and was found to be not less than 95% pure.

Benzenediazonium bisulfate and the analogous salts derived from the three aminobenzoic acids were prepared by a modification of methods used previously.[6,8] The crudes were partially dissolved in water and reprecipitated by the addition of methyl alcohol, then ether. Stock solutions were 0.01 *M* and were preserved as described above for chloride solutions. Sulfate analyses[9] showed these solids to be pure diazonium bisulfates.

All other materials used in the preparation of solutions for polarographic or coulometric analysis were examined to insure freedom from reducible impurities in the potential range studied.

A Sargent–Heyrovsky Model XII polarograph was employed. Capillary constants and other similar data are included in Table I. Coulometric analysis was performed at 0° by conventional procedures.[10] Pre-electrolysis of the supporting electrolyte and buffer solutions prior to the addition of the diazo solution was performed at a potential 0.1 to 0.2 v. more negative than that used in the actual coulometric analysis.

Results

Table I contains representative data obtained with the four diazo compounds under sixty diverse sets of experimental conditions. Figs. 1–3 indicate the quality of the curves obtained. Concentrations of 2 millimolar or less were used because at higher concentrations the maximum associated with the first wave could not be suppressed, intermediate waves appeared and the capillary activity of the diazo compound gave rise to minima (Fig. 3e). Streaming of the dropping electrode at −0.4 v. *vs.* S. C. E. occurred in 5×10^{-2} *M* solutions.

The important observable features of the polarographic results may be summarized conveniently as follows:

(1) All four substances exhibited two waves in the range from 0 to −1.5 v. *vs.* S. C. E. In selected cases more negative potentials were examined but no additional waves were detected prior to discharge of the supporting electrolyte. The first wave exhibited a pronounced maximum which was best suppressed by gelatin.

(2) Diffusion currents for the total second wave decreased rapidly in the higher *p*H range. Diazotized aniline, *m*- and *p*-aminobenzoic acids showed little or no diffusion currents above *p*H 11.5; diazotized anthranilic acid retained the typical two-wave curve as high as *p*H 12.5.

(3) At a given *p*H, or over a limited range of *p*H, diffusion currents for each wave were proportional to concentration of diazotized aniline, the only compound so examined (Fig. 3).

(4) Half-wave potentials for each wave varied

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[1] Presented before the Division of Organic Chemistry at New York, N. Y., September, 1947, and Atlantic City, September, 1949.

[2] Atkinson, Morgan, Warren and Manning, THIS JOURNAL, **67**, 1513 (1945).

[3] (a) Hodgson, *J. Chem. Soc.*, 348 (1948); (b) Waters, *ibid.*, 266 (1942); Saunders and Waters, *ibid.*, 1154 (1946); Hey and Waters, *ibid.*, 882 (1948); (c) Cowdrey and Davies, *ibid.*, S48 (1949).

[4] Atkinson, *et al.*, Buffalo meeting of the American Chemical Society, September, 1942.

[5] After this work had been completed, Elofson and Mecherly, *Anal. Chem.*, **21**, 565 (1949), stated that while diazo compounds exhibited a complex behavior, their polarographic activity at −0.3 v. *vs.* S. C. E. could be used in an amperometric titration.

[6] Pray, *J. Phys. Chem.*, **30**, 1477 (1926).

[7] Kolthoff and Sandell, "Textbook of Quantitative Inorganic Analysis," The Macmillan Co., New York, N. Y., 1947, p. 571.

[8] Hodgson and Mahadevan, *J. Chem. Soc.*, 325 (1947).

[9] Ref. 7, p. 340.

[10] Lingane, THIS JOURNAL, **67**, 1916 (1945).